



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/AU87/00371  <b>(22) International Filing Date:</b> 5 November 1987 (05.11.87)  <b>(31) Priority Application Number:</b> PH 8813  <b>(32) Priority Date:</b> 5 November 1986 (05.11.86)  <b>(33) Priority Country:</b> AU  <b>(71) Applicant (for all designated States except US):</b> GEORGE WESTON FOODS LIMITED [AU/AU]; 40 Marcus Clarke Street, Canberra, ACT 2600 (AU).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> TOMLINSON, John, David [AU/AU]; 12 Miowera Road, Nor- thbridge, NSW 2063 (AU). ROBERTSON, Jennifer, Anne [AU/AU]; 58 Findlay Avenue, Roseville, NSW 2069 (AU). THOMSON, William, Kenneth [AU/AU]; 84 Malton Road, Beecroft, NSW 2119 (AU).		<b>(74) Agent:</b> WATERMARK; Suite 6, Floor 16, Town Hall House, 456 Kent Street, Sydney, NSW 2000 (AU).  <b>(81) Designated States:</b> AT (European patent), AU, BE (Eu- ropean patent), CH, CH (European patent), DE (Eu- ropean patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), US.  Published With international search report.
<b>(54) Title:</b> NOVEL IMPROVERS FOR FLOUR AND YEAST RAISED BAKED GOODS  <b>(57) Abstract</b>  A group of novel natural improvers for yeast raised baked goods, including products derived from the residues of ethanolic and other fermentations of micro-organisms (e.g., lactic). The active components extracted from these residues are the natural electron passing compounds of the respiratory chain and intermediary metabolism as it is linked to energy metabolism. The compounds include nicotinamide adenosine dinucleotide, and its phosphate, flavin adenosine nucleotide flavin mononucleotide, the ubiquinones, cytochromes etc., and the components of these substances e.g. nicotinamide, rib- oflavin, etc., and their derivatives. Significant advantages to the quality of the final yeast raised baked goods are conferred by these natural oxido-reductants and coenzymes, particularly in those not containing traditional or "chemical" oxidising-reducing agents or emulsifiers. "Chemical" oxidising-reducing agents refer to chemicals such as potassium bromate, sodium metabisulfite, azodicarbonamide etc., which are non-biological. "Biological" or "natural" oxidising-reducing agents refer to substances which occur naturally in food and other biological products irrespective of whether they may be chemically synthesised e.g., ascorbic acid, L-cysteine hydrochloride. "Natural" oxidising-reducing agents further imply that the substances may be extracted from naturally occurring biological substances including waste products. The addition of such natural improvers in yeast raised baked goods makes it possible to eliminate the need to use chemical additives in these products and also to partially or fully replace commonly used emulsifiers, e.g., sodium stearoyl lactylate and enzymes, e.g., proteases, phospholipases etc., from the mix when chemical oxidising-reducing agents are used.		

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"NOVEL IMPROVERS FOR FLOUR AND YEAST RAISED BAKED GOODS"TECHNICAL FIELD

5 The present invention relates to the use of highly purified natural improving agents in the production of bread and other yeast raised baked goods. It also relates to the recovery of extracts containing highly purified improving agents for use in bread and other yeast raised baked goods, which are derived from the residues of "waste" products of fermentation and food production industries and to the use of these highly concentrated food quality improving agents in the production of bread and other yeast raised baked goods.

BACKGROUND ART

15 It is well known that the making of bread uses mechanical operations such as kneading the dough, dividing the dough into pieces, and moulding into a predetermined form before proofing and baking. The physical properties of the dough, such as elasticity, extensibility, non-stickiness and ability to be moulded are dependent on a combination of factors including the quality of the flour, the quality of the gluten, and/or the presence of other food additives. In addition the quality of the final baked product as judged by parameters such as crumb softness, volume, texture, taste, mouthfeel etc., is equally dependent upon the above physical properties and is affected by both the chemical and is biological parameters.

20 Using traditional methods of breadmaking, the period of bulk fermentation is important. It is a resting period, during which time the yeast ferments, interacts with the gluten in the dough, and the dough itself changes from a rough, dense mass with poor gas retention and lacking extensibility, into a smooth extensible dough with good gas retaining properties. Such properties are essential to the production of a loaf with good volume, soft yet resilient with fine crumb cell structure. Any method of bread making which omits bulk dough fermentation can only succeed in

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producing bread of good quality if it brings about the same physical and chemical changes by alternative means.

To reduce the bulk of flour requiring long contact with the yeast, a sponge and dough system may be employed in which between 25 and 80% of the flour is fermented with the yeast for 2 to 6 hours. The remainder of the flour is then mixed with the sponge during what is called the "dough" stage. Further variations to this procedure include "flour brews" or "water brews" where the yeast is exposed to a portion of the flour with some yeast foods, e.g., sugar or to a liquid containing yeast nutrients which is easily stirred.

All processes which require extended fermentations (dough, sponges or brew) are wasteful of time and space in the bakery. Modern baking technology, particularly in the U.K. and Australia, utilises high energy dough mixing including the Chorleywood process combined with a short bulk dough fermentation period which is referred to as a "straight" or "instant" dough.

In order to maximise the effect of gluten in the dough structure and to obtain maximum quality parameters as assessed by the indices of taste, flavour, crumb structure, crumb softness, volume etc., (as previously stated), a variety of improving agents are employed. These improving agents include chemical oxidising and reducing agents such as potassium bromate and iodate, sodium metabisulfite as well as biological oxido-reductants such as ascorbic acid and the amino acid L-cysteine. A variety of other improving agents are also used. Emulsifiers which act on the gluten such as sodium stearoyl lactylate, polysorbate 60 and the diacetyl tartaric acid esters ('DATA') are frequently used to improve quality parameters as described and also to retard the staling process. Enzymes such as  $\alpha$ -amylases, proteases, phospholipases are used to modify either gluten or flour components. Such agents are all of biological origin and as such may be considered to be "natural".

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Traditionally produced breads using longer bulk fermentations in the absence of, or in the presence of very small quantities of, chemical or other added improvers exhibit desirable quality characteristics including flavour, crumb structure, crumb softness and resistance to staling. Breads derived from the Chorleywood or other instant dough processes require the addition of improving agents to reach an acceptable quality with respect to volume, crumb structure, mouthfeel and staling characteristics but often lack acceptable sensory and olfactory parameters.

Recently, fermented flour and whey additives have become available, e.g., Empruv, Fermitech etc, which claim to cause an improvement in the quality of doughs to which they are added including sensory and olfactory parameters.

These manifestations are obvious to those persons with ordinary skill in the art. Coupled with our observations that the greater the contact time (from 2 to 24 hours) between the yeast and dough, sponge, brew or ferment system, the better leavening, texture, flavour and resistance to staling in the bread or other yeast raised baked goods, led us to postulate that a metabolite or group of metabolites formed during the fermentation acted upon the gluten and then on the physical properties of the dough in a beneficial manner. Historically, it has been known to those skilled in the art that breads made with the liquors of microbial fermentations have a lighter, softer texture and are more resistant to staling than conventional breads (Tannahill R. 'Food in History' Stein & Day 1972). We postulate that the causative metabolite or group of metabolites is common to each of the described systems. The fact that such metabolites are biological in origin and are derived by natural processes upon food ingredients in day to day use in yeast raised baked goods is significant. There is a groundswell of public opinion and legislative action in many countries, including Australia and the United Kingdom, to limit food additives to strictly defined substances,

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which must be disclosed on labels ('Milling' June 1986).  
The potential exists therefore for the application of  
functional natural compounds in the food industry in general  
and in the production of yeast raised baked goods in  
particular.

DISCLOSURE OF THE INVENTION

It is an object of the present invention to  
provide novel food quality improving agents for use in the  
production of bread and other yeast raised baked goods.  
These improving agents are highly purified substances,  
including nicotinamide adenosine dinucleotide, flavin  
mononucleotide, flavin adenosine dinucleotide, the  
cytochromes, and the ubiquinones, derived from biological  
systems; the improving agents also include components and  
derivatives of these substances, e.g., nicotinamide,  
riboflavin etc.

It is a further object of this invention to  
provide novel food improving agents for use in the  
production of bread and other yeast raised baked goods which  
are concentrated extracts from spent fermentation residues.

It is another object of this invention to provide  
novel food quality improving agents, which will improve the  
quality of bread and other yeast raised baked goods by  
complete replacement of, or reduction in, the levels of  
chemical improving agents, such as potassium bromate, sodium  
metabisulfite, sodium stearoyl lactylate or the diacetyl  
tartaric esters of monoglycerides or distilled  
monoglycerides, which will permit a reduction in bulk  
fermentation time without the concomitant loss of quality  
parameters of flavour, texture, resistance to staling etc.

It is yet a further object of this invention to  
provide novel food quality improving agents which will  
permit a reduction in the amount of yeast required for the  
manufacture of yeast raised baked goods.

It is yet another object of this invention to  
provide a process for recovery from fermented wastes or

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fermentation residues, e.g., from breweries, wineries, starch plants and dairies of highly concentrated natural novel improving agents for yeast raised baked goods.

These and other objects of the invention will be apparent from the following further disclosure of the invention.

According to one of its aspects the present invention provides food quality novel natural improving agents for yeast raised baked goods, including any one of, or any combination of, natural electron transfer biological oxido-reductants including oxidative chain phosphorylation components or derivatives thereof, including for example:-

NAD nicotinamide adenosine dinucleotide

NADP nicotinamide adenosine dinucleotide  
phosphate

FMN flavin mononucleotide

FAD flavin adenosine dinucleotide

Ubiquinones - i.e., the coenzymes Q

Cytochromes

and other components of the electron transport chain.

According to another aspect of the invention there is provided a process for recovery of concentrated extracts of such oxido-reductants or electron passing compounds of the respiratory chain and intermediary metabolism from fermentation residues or "wastes", the process comprising subjecting such residues or wastes to treatment including cell disruption, heat treatment, clarification, purification, concentration, dehydration and stabilization of the extract.

According to a further aspect the present invention relates to the use of such oxido-reductants or electron passing compounds, either singularly or in any combination as food quality improving agents, especially in the production of yeast raised baked goods as a full or partial replacement of, or in addition to, chemical improving agents.

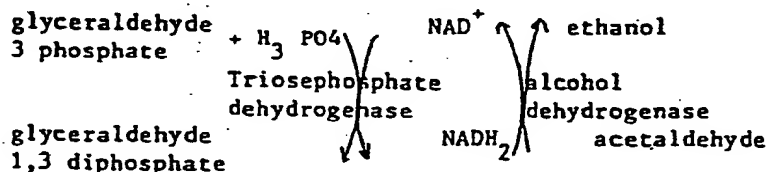
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These compounds may be used either in a highly purified state or as components of concentrates and extracts contained in or derived from spent residues from the fermentations of yeasts (especially of the Saccharomyces genus) as for example from beer, wine and other alcoholic beverages, and also from the spent residues from fermentations of other micro-organisms, e.g., Acetobacter, Lactobacillus, Aspergillus, Leuconostoc and Streptococcus species. Also, starch plant residues and dairy residues.

Generally, the concentrations of the electron-passing compounds employed are at least 0.001% by weight, normally expressed as a percentage of the weight of flour in the mixture.

NAD and NADP are natural oxido-reductant present in most biochemical systems. They are coenzymes for a wide variety of enzymes. The NAD dependent enzymes exhibit several modes of action, four of which are discussed herein. First, the NAD dependent dehydrogenases catalyse the oxidation of alcohols, aldehydes,  $\alpha$ - and  $\beta$ -hydroxy carboxylic acids and  $\alpha$ -amino acids. The nucleotides themselves readily accept electrons from a reduced substrate or donate electrons to an oxidised substrate in a coupled reaction such as

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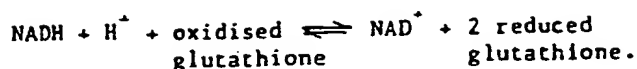


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Secondly the nicotinamide nucleotides function in reduction of the flavin coenzymes. These provide the link in the electron transport chain between NAD and FMN or FAD. An example is the reduction of oxidised glutathione by glutathione reductase.

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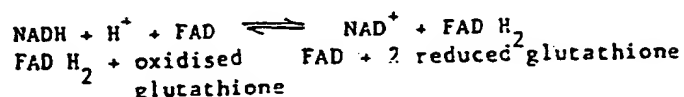




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Glutathione reductases are enzymes which contain FAD as a prosthetic group. The FAD reacts with the NADH as shown

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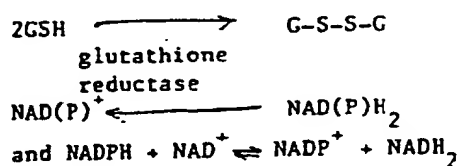
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Thirdly, the nicotinamide nucleotides provide a source of electrons for the hydroxylation and desaturation of both aromatic and aliphatic compounds as for example in lipid metabolism. A further function of NAD is in the repair mechanism of DNA, catalysed by DNA-ligase.

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As is indicated above, NAD is an end product of alcoholic fermentation. It is also one of the end products of intermediary metabolism. Historically, its function in yeast raised baked goods depends upon an interaction with the glutathione reductase system and the oxidation of reduced glutathione. While glutathione reductase has been reported to have specificity for NADP, NAD can collect H atoms from substrates acted upon NADP linked dehydrogenases.

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30

Traditionally, the rheological properties of doughs have been considered to be related to the total number of and distribution of disulfide bonds in gluten (Bloksma, 1972). The thiol/disulfide interchange represents a dynamically changing system dependent on the number, and distribution of these functional groups, i.e., either intermolecular or intramolecular, as well as the

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oxidation-reduction parameters in the system.

However, the historical views of the complex processes occurring during dough fermentation are surprisingly restricted. While they encompass reactions which are likely to occur in a wheat flour/water dough where the glutathione and NADP sources are most likely the germ, they do not explain or appear to take into account reactions occurring between gluten and yeast during fermentation.

Yeast is a living organism. As such it requires nutrients during its logarithmic growth phase. These are provided from the constituents of the flour and/or by the addition of yeast foods e.g., sugar, nitrogen sources and trace elements. Secondly, yeast cell membrane constituents come into direct contact with the gluten in the dough. The possibility exists for interactions between the constituents of the yeast membrane and those of the gluten. Thirdly, living cells have a finite life span. In longer fermentation doughs the percentage of damaged or dead yeast cells will be higher than in short time doughs. Consequently there will be released into the fermenting dough, intracellular enzymes, coenzymes and other metabolites.

It has been surprisingly discovered by us, that the interactions between the individual components of the electron transport chain and the related compounds of intermediary metabolism with gluten and/or flour vary from, and are inconsistent with, the historical view. According to the present invention it can be demonstrated that there is also a direct action of these substances on the gluten with does not appear to involve either glutathione reductase or thiol/disulfide interchange.

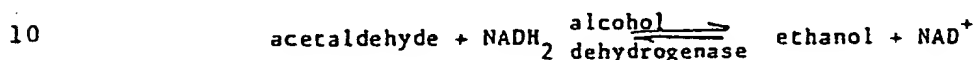
According to the present invention it can be shown that the spent yeast and micro-organisms of fermentations are a rich source of such metabolites. Fresh Baker's yeast is the usual source of commercial NAD and related metabolites, although patents describing its

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production and purification from aerobic culturing of micro-organisms are extant (e.g., see USP 3,705,080; USP 3,705,081; USP 3,708,394 and USP 3,709,786).

5 yields of NAD and related metabolites from fresh baker's yeast are small, while those from the aerobic culturing of micro-organisms are higher. Irrespective of method of production, costs of the final products are high.

Since NAD is produced as an end product of ethanolic and lactic fermentations:



15 it has now been surprisingly discovered according to the invention that its concentration in the end products of alcoholic and lactic fermentations and in the microbial residues from such fermentations is an order of magnitude greater than in baker's yeast, thus making its recovery commercially viable.

20 NAD is estimated using the enzyme catalysed oxidation of ethanol to acetaldehyde at pH 9.0. The reaction mixture consists of 100mM phosphate buffer pH 9.0, ethanol 170mM, alcohol dehydrogenase (Boehringer Mannheim) 20 units/mL in a total reaction mixutre of 2.5mL. The  
25 reaction is carried out at 37°C in a cuvette with a 1cm light path, and the change in absorbance at 340nm is used to calculate the concentration of NAD.

As is known to those skilled in the art of making yeast raised baked goods, those breads in particular which  
30 are made using beer or wine to replace some or all of the water have a different flavour, texture, mouthfeel and resistance to staling to those made using only water, ('Milling', June 1986). Until the present invention, the components within these liquors which were responsible had  
35 not been identified. Further, inter-relationships between these active components and other improver ingredients,

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e.g.,  $\alpha$ -amylase, ascorbic acid etc., in breads and yeast raised baked goods had not previously been described or understood.

5 NAD and the related compounds according to the present invention act as strong oxidising agents on gluten, either alone or when in combination with flour, but at the same time increase extensibility of the dough. In the presence of ascorbic acid, the oxidising power of the NAD and related compounds is reduced without a concomitant  
10 significant reduction in extensibility of the dough. In breads and yeast raised baked goods, the presence of ascorbic acid and  $\alpha$ -amylase may be beneficial to produce maximum advantage with respect to volume; texture; mouthfeel and other physical parameters. According to the present  
15 invention some embodiments of the improving agent may also comprise one or more additives selected from  $\alpha$ -amylase, ascorbic acid and phospholipase A and/or phospholipase D, in an appropriate effective amount, usually at levels of not less than 0.0001% by weight.

20 Sources of NAD and Related Compounds.

Broadly speaking, natural sources of NAD, FAD, FMN, NADP, the ubiquinones are the end products of microbial fermentations. Examples of possible sources include:-

- (i) yeast residues from breweries
- 25 (ii) spent grain from breweries
- (iii) yeast residues or lees from wineries
- (iv) residues from micro-organisms from lactic fermentations
- (v) residue liquors from lactic fermentations, e.g.,  
30 starch plants or dairy residues.

Examples given relate to extensive tests and processing of yeast residues from breweries but also include those from wine lees. As supplied the yeast residue is a thick cake, still biologically active but with a high moisture and  
35 ethanol content. The yeast cells contain large intracellular concentrations of NAD, in addition to other

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components of the electron transport chain and intermediary metabolism as previously cited.

FULL DESCRIPTION OF THE EMBODIMENTS

5 The preferred method for the processing of the residues from fermentation systems in order to obtain highly purified extracts and residues for use in yeast raised baked goods may comprise some of or all of the following processes in any combination or order:-

10 Cell disruption  
Heat treatment  
Clarification  
Purification  
Concentration  
Dehydration.

15 Cell Disruption

High pressure homogenisation and freezing (or heating) of the fermented residue and residue products of fermentation are two of the methods which have been employed according to the invention to disrupt and/or weaken the cell walls of the yeast. Other methods may include for example, 20 extrusion, colloid milling, microwaving, lysozymes, solvent extraction, high pressure spray drying, vibration ball milling, treatment with ultrasonics and grinding. Cell disruption may be followed by inactivation of deleterious enzyme and other catalytic systems by appropriate physical and chemical change.

25 Heat Treatment

A number of techniques may be employed to inactivate enzyme systems, including elevating or depressing 30 temperatures, adjustment of pH or combinations of these. The fermented microbial residues or the cell disrupted microbial residues are diluted with water to a viscosity approaching that of water. The diluted mixture is subjected to a temperature greater than 45°C but substantially less than 200°C for a period of not more than 30 minutes. This 35 is of extreme importance not only as a consequence of

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inactivating deleterious enzyme systems, but also because of the final yield and quality of the NAD and the other oxido-reductants and metabolites hitherto described. This type of process not only disrupts the cells in previously untreated microbial fermented residues but also permits the release into the medium of intracellular pools of the NAD and other oxido-reductants. It will be appreciated that inactivation of enzyme systems and cell disruption may be achieved by temperature reduction to below freezing point followed by thawing. Moreover, a combination of adjustments of pH and temperature is to be preferred. NAD is unstable in alkaline solutions and furthermore is unstable at low pH and high temperatures. Preferred working conditions for maximum recovery of NAD are within narrow pH limits known to those skilled in the art.

#### Clarification and Filtration

Clarification of the cell disrupted enzyme inactivated fermented waste to remove cell debris from the extract containing the NAD and other metabolites is successfully achieved by centrifugation or filtration, e.g., the use of Sharples decanter or starch bed filtration.

Other means of clarification will occur to those skilled in the art in the light of the present disclosure, as for example the use of filter aids such as diatomaceous earth, vacuum drum filtration flocculation, flotation bubbles, pressure candles, enzymes or membranes.

#### Dehydration and Concentration

The present invention relates to a process for producing a highly concentrated extract from one of a group of fermented residues and "waste" residues of fermentations including brewery residues, winery residues, lactic fermentation residues containing the natural oxido-reductant NAD and related compounds previously described. It will be understood by those skilled in the art that several dehydration and concentration techniques may be applied to obtain the highly concentrated dehydrated extract include

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freeze drying, spray drying, drying on carriers, e.g., starch or microwave drying. A highly concentrated liquid extract may be obtained by any or all of a variety of concentration processes including ultrafiltration, reverse osmosis, falling film evaporators, and the like.

Each of these methods of dehydration and concentration can be used on the crude ferment residues or on the extract containing the active principles of the coenzymes, metabolites and oxido-reductants. Freeze drying of the heat treated residue, the heat treated extract and the residual cell debris, have provided free-flowing products. Dehydration of both the heat-treated residue and extract provided products which, when baked, results in significant improvement in volume, crumb texture and retardation of staling in yeast raised baked goods. Addition of the crude residue or the heat treated extract to starch in a 1:1 ratio permitted the dehydration to be carried out using a fluidised bed drier. The final product required grinding but provides increases in NAD and in volume and improved textural characteristics in bread and yeast raised baked goods.

Notwithstanding the fact that fermented yeast and microbial residues contain high concentrations of endogenous non-protein, non-starch hydrophilic colloids and carbohydrates, the products herein described can be successfully obtained by spray drying. The preferred method uses an inlet temperature into the spray drier of between about 180°C and 300°C and an outlet temperature of between 70°C and 120°C. Advantages of lower inlet and outlet temperatures on the performance of the final spray dried concentrate in yeast raised baked goods will be obvious to those skilled in the art in view of the present disclosure.

The present invention relates to the novel use of naturally occurring oxido-reductants, metabolites and coenzymes in bread and other yeast raised baked goods as in adjunct to currently available "chemical improvers", or as a

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partial or complete replacement of such improvers. It will of course be understood that "chemical" refers to improvers such as potassium bromate, potassium iodate, azodicarbonamides, sodium metabisulfite, emulsifiers, 5 detergents, etc., which are not naturally occurring. Biological or natural substances refer to those which occur naturally in foods and biological products irrespective of whether they may be chemically synthesised, e.g., ascorbic acid, L-cysteine or phospholipases.

10 The following specific examples illustrate some aspects of the present invention. They are set forth by way of illustration and teaching only, and are not to be construed as limiting on the scope of the present invention.  
Example 1.

15 The following procedure represents a preferred process for obtaining a highly concentrated extract from semi-solid brewery yeast residues. 20 litres of semi-solid waste yeast was diluted 1:1 with water and autoclaved at 120°C for 10 minutes in order to effect both cell disruption 20 and enzyme inactivation. The resulting mixture was rapidly cooled to 4°C and acidified to pH of 4 with glacial acetic acid. The cell debris was removed from the soluble extract containing the NAD, and other metabolites by centrifugation using a g force of 5000 for 10 minutes. It is to be noted 25 that several of the sequential procedures described can be incorporated into fewer steps. The clear liquor from the centrifugation step, was frozen and subjected to freeze drying. The final dehydrated product was ground and mixed with and equal mass of wheaten starch.

30 Example 2.

The following study was undertaken to determine the effect of the addition of the product from Example 1 into a variety of breads made using a range of Harvest and Cereform bread improvers. Loaves of bread were made in the 35 following manner:-

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- Mixing : 3.75 minutes for white loaves and 4 minutes for wholemeal
- Bulk fermentation : 20 minutes at 30°C
- Bench time : 10 minutes
- 5 Moulding : 400g per loaf
- Proofing : 45 minutes at 38°C and 70% relative humidity
- Baking : 210°C for 25 minutes

The raw materials used for each loaf are set forth in

10 Tables 1a and 1b.

Table 1a Raw Materials (part by weight)

V8

	<u>Ingredients</u>	<u>White Control</u>	<u>White Natural</u>
	Supreme ++ Flour	1000	1000
15	Salt	20	20
	Soya Flour	10	-
	Yeast*	12	12
	Improver V8	2.5	-
	Improver Natural**	-	23

20

Table 1b Formulation (parts by weight)

	<u>Ingredients</u>	<u>Wholemeal Control</u>	<u>Wholemeal Natural</u>
	Supreme++ Flour	820	820
25	Harvest* Meal	180	180
	Salt	20	20
	Gluten	40	40
	Yeast'	14	14
	V8*	2.5	-
30	Natural Improver**	-	23

\* Harvest is a registered trademark of George Weston Foods Ltd, Sydney, N.S.W. Australia.

' Yeast is Mauripan Active Dried Yeast produced by Mauri Foods, Camellia, N.S.W. Australia.

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\*\* Natural improver is produced by Cereform, Wetherill

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Park, N.S.W. Australia, containing soya flour, soya lecithin,  $\alpha$ -amylase (cereal) and ascorbic acid.

- ++ Supreme flour is a high protein baker's flour produced by N.B. Love, Enfield, N.S.W. Australia. V8 is a chemical based improver containing potassium bromate, sodium metabisulfite, but no emulsifiers.

Following baking, the loaves were cooled to room temperature, volumes were measured using displacement of rapeseed, sliced, sealed in polyethylene bags and stored at room temperature for several days. Staling measurements were performed using an Instron 4301 Universal Testing Machine. Measurements were carried out on minimum 30cm slices of the loaf, at six sites across the loaf, excluding the heel. Mean values of force per unit area to compress the centre of individual slices of bread to a depth of 10mm were calculated. Results for volume and staling are shown in Table 2.

Table 2. Effect of Example 1 Product on Loaf Volume.

	<u>Type of Loaf</u>	<u>Volume</u>	<u>Staling</u>
		(ml.)	(Newtons)
	White Control	5430	5.015 $\pm$ 0.245
25	White Control + 0.05% product from Example 1 on a flour weight basis	5740	4.49 $\pm$ 0.260*
	White Natural	5220	5.582 $\pm$ 0.226
	White Natural + 0.05% product from Example 1	5440	4.841 $\pm$ 0.278*
30	Wholemeal Control	5500	4.447 $\pm$ 0.419
	Wholemeal Control + 0.05% product from Example 1	5760	3.971 $\pm$ 0.245
	Wholemeal Natural	4960	4.859 $\pm$ 0.214
35	Wholemeal Natural + 0.05% product from Example 1	5510	3.971 $\pm$ 0.194

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Representative volumes for 3 loaves baked on the same day are given. Values which differ from controls by more than 100 units are significant.

5           \* Significant at the  $P < 0.05$  level. The lower the value the softer the bread.

10           It can be seen from the representative results of Table 2 that replacement of chemical improvers such as V8 with natural improvers results in a significant reduction in loaf volume. Addition of the product from Example 1 at the preferred usage rate of 0.05% based on the flour or flour/meal content of the system provided an increase in volume back to volumes obtained with chemical improvers alone. When the product from Example 1 was added to systems  
15           containing chemical improvers, it provided a significant increase in the loaf volume. In addition to the improvement in loaf volume, addition of product from Example 1 at the usage rate of 0.05% based on the flour or flour/meal content of the system, provided a retardation in the staling or  
20           firming characteristics of the loaves. Crumb structure and texture in loaves containing the product of Example 1 were markedly different from those containing only V8 or natural improver. The crumb structure is much finer, cells are small and elliptical in shape. The crumb is more resilient  
25           and possesses an iridescent sheen.

Example 3.

          The following study was undertaken to determine the effect of addition of pure NAD (Boehringer-Mannheim lithium salt 100% pure or sodium salt 98%) into a variety of  
30           breads made using a range of Harvest and Cereform improvers. Loaves of bread are made by mixing raw materials set out in Table 1 and following the same breadmaking process as outlined in Example 2, with the addition of low concentrations of pure NAD in lieu of the processed highly  
35           concentrated extract produced in Example 1. The volumes of the loaves results are shown in Table 3.

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Table 3. Effect of NAD on volume.

	<u>Type of Loaf</u>	<u>Volume</u> (ml.)	<u>Staling</u> (Newtons)
5	White Control	5430	5.015+0.245
	White Control + 0.005% NAD	5690	4.56 +0.312*
10	White Natural	5220	5.582+0.226
	White Natural + 0.005% NAD	5460	4.903+0.188*
	Wholemeal Control	5500	4.447+0.419
	Wholemeal Control + 0.005% NAD	5730	3.715+0.276*
15	Wholemeal Natural	4960	4.859+0.214
	Wholemeal Natural + 0.005% NAD	5450	3.827+0.255*

\* Significant at the  $P < 0.05$  level.

20

As can be seen from the above representative sample, the addition of pure NAD provided an increase in volume as well as a retardation in the onset of staling. Crumb structure and texture replicated that seen in the loaves baked using the highly concentrated extract of Example 1.

25

Example 4.

30

In order to determine the interaction between the product of Example 1 or pure NAD with ascorbic acid, loaves of bread were made by mixing the raw materials as set out in Table 1 and using the same breadmaking process of Example 2, using varying levels of:

35

- a) the extract obtained from the process of Example 1 and ascorbic acid,
- b) Pure NAD and ascorbic acid.

Representative results for wholemeal are shown in Table 4.

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Table 4. Interaction between NAD and Ascorbic Acid.

		<u>Loaf Volume</u>			
% Ascorbic Acid		0	0.0035%	0.007%	0.01%
5	Wholemeal	6390	6430	6730	6960
	Wholemeal + 0.005% NAD	6150	6640	6930	7150
	Wholemeal + 0.05% product				
	of Example 1	6140	6670	6970	7180

10                   Loaf volumes quoted are representative only and vary from day to day. Results in Table 4 show the NAD and the product from Example 1 interact with ascorbic acid in the dough. Unless ascorbic acid is present the NAD and product from Example 1 exert such a strong effect on the  
15                   dough, that there is a resultant reduction in loaf volume.

Example 5.

                  The following example demonstrates the interaction between  $\alpha$ -amylase and the products described in  
20                   Examples 3 of the present invention. Loaves of bread were baked using the raw materials as set out in Table 1 and the breadmaking process of Example 2. The source of  $\alpha$ -amylase was cereal, and the activity was determined using the Phadebas (TM) (Pharmacia) amylase activity test.

25                   Representative results are shown in Table 5 for wholemeal loaves.

Table 5. Effect of  $\alpha$ -amylase on loaf volume.

		<u>Loaf Volume</u>			
* $\alpha$ -amylase addition		0	0.25%	0.5%	0.75%
30	Wholemeal + Natural Improver	4800	4910	4930	5230
	Wholemeal + NAD 0.005% +				
	Natural Improver	4880	5060	5220	5430

35                   \*  $\alpha$ -amylase added as diastatic malt flour containing not more than 600 SKB units per g. % representation of the flour.

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The above clearly illustrates the desirability of using  $\alpha$ -amylase in conjunction with the products claimed in the present invention, in improvers.

5     Example 6

      The following example illustrates the effects of flavin adenosine dinucleotide (FAD) as an improving agent with similar properties to those of NAD and the highly concentrated extract of Example 1. FAD was added to the  
10    loaves at levels up to 0.005%, the loaves were baked according to the scheme in Example 2, volumes were measured as described in Example 2. Results from this example indicate that FAD, when added to bread doughs at the level  
15    of 0.005%, causes an increase in loaf volume of 3% (significant at the p 0.05 level) and crumb structure and texture similar to that of the product from Examples 2 and 3.

Example 7

20       The following example illustrates the effects of flavin mononucleotide (FMN) as an improving agent with similar properties to those of NAD and the highly concentrated extract of Example 1. FMN was added to the  
25    loaves at levels up to 0.005%. The results from these tests show in Table 6 that the 0.005% level of addition of FMN is preferred. Crumb structure and texture was similar to that of loaves baked using the products from Example 1 and Example 3.

30    Table 6   Effect of FMN addition on Loaf Volume.

	<u>Loaf Volume</u>
White + Natural Improver	5160
White + Natural Improver	5010
+ 0.001% FMN	
White + Natural Improver	5340
35       + 0.005% FMN	

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Example 8

This study was undertaken to determine the effect of binding free sulfhydryl groups in flour with FAD and then adding NAD into the improver system. It is known that FAD binds to free sulfhydryl groups and that in the Supreme Flour used in these Examples the concentration of these groups is approximately 3 mg %. Accordingly the FAD was added at 0.003% and NAD added to the ingredients listed in Table 1 at the rate of 0.0025% on a flour basis.

From the results in Table 8 it is obvious that having removed the free sulfhydryl groups, the NAD is free to interact at other parts of the gluten in the flour and thus provide an increase in loaf volume and concomitant improvement in crumb structure and texture.

Example 9

This example describes a preferred process for the recovery of a highly purified extract from either bentonite wine lees or wine lees. Bentonite is added to wine lees as a filter aid at the rate of 5%. The lees are autoclaved for 10 minutes at 120°C, cooled rapidly to 4°C and filtered under vacuum. The filtrate is frozen to -20°C and freeze dried. The final dried product is ground and mixed with an equivalent mass of wheaten starch.

Example 10

This example pertains to the use of the product from Example 9 as an improving agent in yeast raised baked goods. Loaves of bread were formulated using the raw ingredients of Table 1 and baked according to the description of Example 2. Volumes of the loaves were measured by rapeseed displacement and recorded. Crumb structure and texture were noted.

Table 7.

Effect of Product from Example 9 on Loaf Volume.

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	<u>Volume</u>
-White + Nat. Improver	5140
-White + Nat. Improver	5280
+ 0.025% Product	
5 Example 9	
-White + Nat. Improver	5440
+ 0.05% Product	
Example 9	

10 The results from this study indicate that the product obtained in Example 9 which is a highly concentrated extract from wine lees provides an increase in loaf volume and crumb structure and texture similar to that obtained with pure NAD, FAD, FMN or the product of Example 1.

15 Table 8 Summary of Representative Values for Loaf Volume for Examples 2-9 in White Natural Improver Loaves.

<u>Type of Loaf</u>	<u>Additive</u>	<u>Loaf Volume</u>
	-	5160
20	0.005% NAD	5460
	0.05% Product of Example 1	5400
White +	0.003% FAD	5230
Natural	0.005% FAD	5280
	0.003% FAD + 0.0025% NAD	5380
25	0.001% FMN	5010
	0.005% FMN	5340
	0.05% Product of Example 9	5440

Example 11

30 The following example illustrates the effect of the addition of product from Example 1 to proprietary chemical improvers containing emulsifiers. White and wholemeal loaves were baked as hitherto described using such improvers.

35 Representative values for volume and staling measurements for both white and wholemeal are shown in Table

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9. The results indicate that the presence of the product of Example 1 and emulsifiers in a chemical improver gives significant increase in volume.

5 Table 9

Effect of Emulsifiers

		<u>White</u>		<u>Wholemeal</u>	
		<u>Volume</u>	<u>Staling</u>	<u>Volume</u>	<u>Staling</u>
10	V8	5180	6.855	4690	6.307
	V8 + 0.05% product of Example 1	5380*	5.842*	4980*	5.728*
15	TN80	5430	4.523	5250	4.538
	TN80 + 0.05% product of Example 1	5540*	4.645	5430*	4.508
	NAF	5410	5.241	5430	4.364
20	NAF + 0.05% product of Example 1	5600*	5.003	5550*	4.508

\* Significant at  $P < 0.05$  level.

25 NAF and TN80 are proprietary improvers produced by Cereform, Wetherill Park, New South Wales, Australia, containing potassium bromate, sodium metabisulfite, ascorbic acid, L-cysteine and emulsifiers such as sodium stearoyl lactylate.

30 The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention. All such modifications as would be obvious to one skilled in the art are intended  
35 to be included herein.

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CLAIMS

1. An improving agent for yeast raised baked goods comprising at least one electron-passing compound of the respiratory chain and intermediary metabolism.
2. An improving agent according to Claim 1, wherein said at least one electron-passing compound is/are selected from the group comprising nicotinamide adenosine dinucleotide (NAD), nicotinamide adenosine dinucleotide phosphate (NADP), flavin mononucleotide (FMN), flavin adenosine dinucleotide (FAD), the cytochromes and the ubiquinones.
3. An improving agent according to Claim 1 or Claim 2, wherein said electron-passing compounds are obtained from fermentation systems.
4. An improving agent according to Claim 3, wherein said electron-passing compounds are derived from the residues of ethanolic fermentations.
5. An improving agent according to Claim 3, wherein said electron-passing compounds are derived from lactic fermentations.
6. An improving agent according to Claim 3, wherein said electron-passing compounds are derived from starch plant residues.
7. An improving agent according to Claim 3, wherein said electron-passing compounds are derived from the production of beer.
8. An improving agent according to Claim 3, wherein said electron-passing compounds are derived from the production of wine.
9. An improving agent according to Claim 3, wherein said electron-passing compounds are derived from whey or other dairy product production residues.
10. An improving agent according to Claim 1 or Claim 2, further comprising ascorbic acid.
11. An improving agent according to any one of Claims 1, 2 and 10, further comprising  $\alpha$ -amylase.

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12. An improving agent according to any one of Claims 1, 2, 10 and 11, further comprising phospholipase A and/or phospholipase D.

13. An electron transfer oxidoreductant compound selected from the group comprising NAD, NADP, FAD, FMN, the cytochromes and the ubiquinones, when used as an improving agent for yeast raised baked goods.

14. The use of any of the natural electron-passing oxidoreductant compounds of the respiratory chain and intermediary metabolism, either alone or in any combination, as food quality improving agents, especially in the production of yeast raised baked goods as a full or partial replacement of, or in addition to chemical improving agents.

15. The use of natural electron-passing oxidoreductant compounds according to Claim 14, wherein said electron-passing oxidoreductant compounds are selected from the group comprising nicotinamide adenosine dinucleotide (NAD), nicotinamide adenosine dinucleotide phosphate (NADP), flavin adenosine dinucleotide (FAD), flavin, mononucleotide (FMN), the cytochromes and the ubiquinones.

16. The use of natural electron-passing oxidoreductant compounds according to Claim 14 or Claim 15 at levels of not less than 0.001% by weight.

17. The use of natural electron-passing oxidoreductant compounds according to any one of Claims 14 to 16, in conjunction with ascorbic acid.

18. The use of natural electron-passing oxidoreductant compounds according to Claim 17 wherein the ascorbic acid is used in an amount of not less than 0.0001% by weight.

19. The use of natural electron-passing oxidoreductant compounds according to any one of Claims 14 to 18, in conjunction with  $\alpha$ -amylase.

20. The use of natural electron-passing oxidoreductant compounds according to any one of Claims 14 to 19, in conjunction with the enzyme phospholipase A and/or

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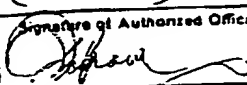
phospholipase D.

21. The use of natural electron-passing oxidoreductant compounds according to Claim 14, to permit the reduction or replacement of chemical substances such as potassium bromate in improvers for yeast raised baked goods.
22. The use of natural electron-passing oxidoreductant compounds according to Claim 14, to permit the reduction or replacement of emulsifiers such as sodium stearyl lactylate in improvers for yeast raised baked goods.
23. The use of natural electron-passing oxidoreductant compounds according to Claim 14, in solution, to permit the partial or total replacement for liquids used in the production of yeast raised baked goods.
24. The use of natural electron-passing oxidoreductant compounds according to Claim 23, wherein said compounds are contained in the discharge liquor from starch plants.
25. The use of natural electron-passing oxidoreductant compounds according to Claim 14, to permit the reduction of the rate of addition of active yeast in yeast raised baked goods.
26. A process for the recovery of concentrated extracts from fermentation residues of natural electron-passing oxidoreductant compounds for use as improving agents for yeast raised baked goods, said process comprising the steps of cell disruption, heat treatment, clarification, purification, concentration, dehydration and stabilisation of said residues.
27. A process for the production of yeast raised baked goods using a dough mixture containing an added improving agent comprising at least one electron-passing compound of the respiratory chain and intermediary metabolism.
28. Yeast raised baked goods when produced by a process according to Claim 27.

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# INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 87/00371

<b>I. CLASSIFICATION OF SUBJECT MATTER:</b> (130-02) classification symbols apply. Indicate sub- According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl. 4 A21D 2/14, 2/18, 2/22, 2/24, 2/26, 2/30, 8/04; C12P 7/26, 17/12, 19/36, 21/02, 25/00		
<b>II. FIELDS SEARCHED:</b>		
Minimum Documentation Searched		
Classification System	Classification Symbols	
IPC	A21D 2/14, 2/18, 2/22, 2/24, 2/26, 2/30, 8/04	
US Cl.	426-18	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched		
AU : IPC as above; Australian Classification 34.6		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
X	Ninth edition of The Merck Index published by Merck & Co., Inc. U.S.A. (1976). See monograph numbers 2788, 2789, 4007, 6170, 6172, 6340, 7993 and 9496 at pages 366, 532, 824, 846, 1064, 1065 and 1263.	(1-12)
X	International Patent Classification Fourth Edition (1984) Volume 3 Section C published by WIPO. See sub-group headings C12P 7/26, 17/12, 19/36, 21/02, 25/00 at pages 180, 181, 182.	(1-12)
X	AU,B, 78444/75 (489076) (SOCIETE DES PRODUITS NESTLE S.A.) 26 August 1976 (26.08.76)	(1,14,16, 21-23,25, 27-28)
X	AU,B, 30657/77 (512853) (STAUFFER CHEMICAL CO.) 24 May 1979 (24.05.79)	(1,3,9,14, 16,21-23,25, 27-28)
X	AU,B, 16272/83 (554037) (NISSHIN FLOUR MILLING CO. LTD) 5 January 1984 (05.01.84)	(1,3,7,14, 16,21-23,25, 27-28)
X	AU,A, 45929/85 (KYOWA HAKKO KOGYO CO., LTD) 13 February 1986 (13.02.86)	(1,3,9-12, 14,16,21-23, 25,27-28)
(continued)		
* Special categories of cited documents: ** -A- document defining the general state of the art which is not considered to be of particular relevance -E- earlier document but published on or after the international filing date -L- document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) -O- document referring to an oral disclosure, use, exhibition or other means -P- document published prior to the international filing date but later than the priority date claimed -T- later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention -X- document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step -Y- document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. -G- document member of the same patent family		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search 11 January 1988 (11.01.88)		Date of Making of this International Search Report (21.01.88) 21 JANUARY 1988
International Searching Authority Australian Patent Office		Signature of Authorized Officer  J. BODEGRAVEN

Form PCT/ISA/210 (second sheet) (January 1985)

BNSDOCID: <WO\_8803365A1\_1>

NZAS-0024016

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	US,A, 3466176 (BUNDUS EVANSTON AND LUKSAS) 9 September 1969 (09.09.69)	(1,3,9,14, 16,21-23,25, 27-28)
X	US,A, 3681083 (EVERSON AND WARREN) 1 August 1972 (01.08.72)	(1,3,5,14, 16,21-23,25, 27-28)
X	US,A, 3689641 (SPANGLER AND WALKER) 5 September 1972 (05.09.72)	(1,14,16, 21-23,25, 27-28)
X	US,A, 3830938 (MORIKAWA et al) 20 August 1974 (20.08.74)	(1,14,16, 21-23,25, 27-28)
X	US,A, 4093748 (AKATSUKA et al) 6 June 1978 (06.06.78)	(1,3,7,8, 14,16,21-23, 25,27-28)
X	US,A, 4243687 (KLINE) 6 January 1981 (06.01.81)	(1,3,14,16, 21-23,25, 27-28)
X	GB,A, 578626 (GREEN) 5 July 1946 (05.07.46)	(1,14,16, 21-23,25, 27-28)
X	GB,A, 2167283 (SIREN) 29 May 1986 (29.05.86)	(1,14,16, 21-23,25, 27-28)
X	EP,A, 97251 (KORN) 4 January 1984 (04.01.84)	(1,3,7,14, 16,21-23, 25,27-28)
X	Derwent Abstract Accession no. 41273Y/23, class D11, SU,A, 528077 (KIEV FOOD IND TECH) 5 October 1976 (05.10.76)	(1,3,14,16, 21-23,25, 27-28)
X	Derwent Abstract Accession no. 83-739811/33, class D11, SU,A, 965415 (KAUN POLY) 18 October 1982 (18.10.82)	(1,3,5,9,14, 16,21-23,25, 27-28)
X	Patents Abstracts of Japan, C77, page 4362, JP,A, 52-130977 (ORIENTAL KOBO KOGYO K.K.) 11 February 1977 (11.02.77)	(1,3,6,14, 21-25,27-28)

Form PCT ISA 210 (extra sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ..... because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claim numbers ..... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically
  
3. ☐ Claim numbers ..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in the international application as follows:

- (1) Claims 1-25 and 27-28 which claim well known oxido-reductants and their use as dough additives.
- (2) Claim 26 which claims a process for recovering extracts from fermentation residues.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1-25 and 27-28

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not advise payment of any additional fee.

Remarks on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON  
INTERNATIONAL APPLICATION NO. PCT/AU 87/00371

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Members	
AU 30657/77	CA 1094875	US 4109025	
AU 16272/83	DE 1547959 CH 473388 NL 6606120 DE 1951185 NL 6915328 AT 4424/72	NL 6504706 DE 1598262 BE 740550 FR 2021985 US 3603344	BE 680102 ES 326468 CH 482968 GB 1237485 DE 2159001
AU 45929/85	FR 2138781 JP 48085706 US 4062016 JP 53102627 DE 3105152 JP 56119120 JP 59145334	GB 1343500 JP 49103381 JP 51091668 JP 53115545 FR 2475838 NL 8100679	US 3804146 JP 50110663 JP 52100187 US 4223679 GB 2072359 JP 58135369
US 3830938	JP 60162116 JP 61175227 JP 51091971 DE 3105152 JP 56165128	AU 46838/85 US 4706617 JP 55007816 FR 2475838 NL 8100679	EP 174149 JP 62173758 US 4062016 GB 2072359
US 4093748	US 4379983 JP 61241417	AU 46838/85 US 4706617	EP 174149
US 4243687	JP 61081519	EP 174149	JP 61157714
GB 2167283	JP 61157715 JP 61164030	AU 46838/85 US 4706617	EP 174149
EP 97251	JP 61164031	US 4706617	EP 174149

END OF ANNEX